CHANGES IN INTENSITY AND SPECTRAL DISTRIBUTION OF FLUORESCENCE

EFFECT OF LIGHT TREATMENT ON NORMAL AND DCMU*-POISONED *ANACYSTIS NIDULANS*‡

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ABSTRACT The intensity of the "steady-state" fluorescence of "aerobic" *Anacystis nidulans* is variable under prolonged illumination with orange (590 mμ) or blue (440 mμ) light for both normally photosynthesizing and DCMU-poisoned cells. In general, orange light illumination causes an increase of the fluorescence intensity followed by a decrease, while blue light causes an increase until a steady level is reached. Poisoned *Anacystis* cells show four to eight times larger changes in fluorescence intensity than the normal cells; the detailed time course of fluorescence changes is also different in poisoned and normal cells. When algae are cooled to −196°C in light, the light-induced changes in the "steady-state" fluorescence disappear in both types of cells. Difference fluorescence spectra, constructed by subtracting the fluorescence spectra taken after 5–15 min of illumination from those after 60–90 min of illumination, show a doublet structure of the difference band with a major peak coinciding with the *Anacystis* emission maximum (685 mμ) and a minor peak located at about 693 mμ.

INTRODUCTION

Steady-state fluorescence spectra of photosynthetic systems have been employed for the characterization of the photosynthetically active pigments and the energy interrelationships between them (1, 2). The quantitative evaluation of steady-state data often is difficult, due to the polyphasic nature of the sample and the variability of the quantum efficiency of fluorescence. Physiological causes (3, 4), such as the age of the organism, or light treatments preceding or during the recording of the spectrum, may have an effect on the fluorescence yield of the system and the spectral distribution of the emitted light.

Fluorescence time-course studies have been, in general, restricted to short times after the onset of excitation; often they have been made with the purpose of correlating early gas exchange kinetic curves with fluorescence induction phenomena. A

* DCMU = 1,1-Dimethyl-3(3′4′-Dichloro-)phenyl urea.

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large body of earlier investigations, with light exposures not exceeding 1 min, have been reviewed by Rabinowitch (5). More recently, technical improvements have permitted the recording of faster emission changes in millisecond range, and attempts have been made to correlate them with the redox reactions occurring between the two photosynthetic systems (6–11). Variations of the fluorescence quantum yield, induced by longtime light treatment have, in general, been much less investigated. Brody and Brody (3) have shown that in *Porphyridium cruentum*, whose pigment ratio has been altered by growing the cells in light absorbed primarily by either chlorophyll *a* (blue) or phycoerythrin (green), preillumination with either blue or green light prior to the recording of the spectrum causes change in both the emission and the excitation spectra, while the absorption characteristics remain unaffected. Ghosh and Govindjee (12) obtained similar results with *Anacystis nidulans* grown in light of different intensity and color. They interpreted their results as indicating decreased efficiency of energy transfer from phycocyanin to the fluorescent chlorophyll *a* component (Chl a_2) and an increased transfer to the weakly fluorescent one (Chl a_1); the former being associated with the photosynthetic system II, the latter with the photosynthetic system I.

In the present investigation, we have studied the effect of prolonged illumination with light absorbed by the photosynthetic systems I and II on the fluorescence properties of normal (nonpoisoned) and DCMU-poisoned *Anacystis nidulans*. Our purpose was to establish the conditions under which the steady-state fluorescence properties of this alga are sufficiently constant with respect to continuous exposure to the exciting radiation, since comparisons of such properties have been used repeatedly in the investigation of the physical status and the energy interrelationships of the photosynthetic pigment complement. Changes induced on the emission and chlorophyll *a* (Chl *a*) excitation spectra as well as time course of the changes have been investigated. Also, an attempt has been made, by using narrow observation band widths, to locate changes in fine structure in the spectral distribution of the emission. It seems that there are two regions (685 and 693 mμ) within the spectral region of Chl *a* fluorescence where changes caused by continuous illumination are peaked.

EXPERIMENTAL

Anacystis nidulans was grown under "white light" of approximately 0.2×10^7 ergs/cm^{-2}/sec^{-1}. The culturing conditions employed were described by Govindjee and Rabinowitch (13). After 3–6 days growth, the cells were transferred to a sodium carbonate-bicarbonate buffer ($[CO_3^{=}] = 0.2$ M, pH = 9.2); the buffer was used to provide CO_2 and to keep the cells from growing. Poisoned samples also contained 5×10^{-5} M DCMU (obtained from E. I. duPont de Nemour & Co., Inc., Wilmington, Del.). The optical density at the Chl *a* red peak of both samples was 0.040 for 1 mm path length. The phycocyanin absorbance exceeded Chl *a* absorbance.

The absorption spectra were measured with a Bausch and Lomb recording spectrophotometer (Spectronic 505, Bausch and Lomb Optical Co., Rochester, N. Y.), equipped with

an integrating sphere attachment. The band width of the measuring beam was 5 mμ. We have used the term "band width" throughout the paper to indicate the width of the band at half its height.

Fluorescence was measured with the spectrofluorometer of Govindjee et al. (14–16). For emission spectra the excitation and treatment light from a 6 v, 18 amp tungsten ribbon lamp went through a large Bausch and Lomb monochromator (600 grooves/mm; dispersion, 3.3 mμ/mm slit width) set at 440 or 590 mμ (band-bandwidths = 20 mμ). The intensities of the 440 and the 590 mμ beams, as measured with Bi/Ag Eppley thermopile (The Eppley Laboratory, Inc., Newport, R. I.), were 1.95×10^4 ergs cm^{-2} sec^{-1} and 3.6×10^4 ergs cm^{-2} sec^{-1}, respectively. The fluorescence spectrum was scanned with an identical monochromator, with measuring band widths of 2.5 mμ for 590 mμ excitation and 3.3 mμ for 440 mμ excitation. The signal, detected by an EMI 9558B photomultiplier (Electra Megadyne Inc., New York, N. Y.), was amplified by a Keithley microvolt ammeter (Keithley Instruments, Cleveland, Ohio, model 150A), and recorded on a Brown recorder (Brown Instrument Division, Minneapolis-Honeywell Reg. Co., Philadelphia, Pa.; model Y153X12(X)-X-(9)-A6(v). The recorded emission spectra were corrected for the wavelength dependence of the photomultiplier sensitivity and of the transmission efficiency of the monochromator. No corrections for the reabsorption of fluorescence were applied because the fluorescence was excited and collected from the same surface and because the optical density of the sample was very low (0.040 for 1 mm path).

For excitation spectra, the measuring monochromator was set at 745 mμ (20 mμ band width) while the exciting monochromator scanned the spectrum with a bandwidth of 5 mμ. Light treatment was provided from a second tungsten lamp; this beam was collimated and passed through a water (5 cm) filter and a Farrand (Farrand Optical Co., Inc., New York, N. Y.) interference filter having a maximum (46%) transmission at 685 mμ and a band width of 11 mμ. The intensity of this beam, at the sample, was 6.46×10^4 ergs cm^{-2} sec^{-1}. The recorded excitation spectra were normalized over a constant incident quantum flux at each wavelength.

The time course of fluorescence was measured at 685 and 650 mμ, with band width of 25 mμ for 440 mμ excitation and treatment (intensity = 1.95×10^4 ergs cm^{-2} sec^{-1}) and 6.6 mμ for 590 mμ excitation and treatment (intensity = 3.6×10^4 ergs cm^{-2} sec^{-1}). The excitation and treatment bandwidths were 20 mμ. Balzer's (Geraetebauanstalt, Balzers, Fürstentum, Liechtenstein) neutral density filters were used for reducing the exciting intensity. Time course data are expressed as the ratio F_t/F_3, where F_t is the intensity of emission after time t of light treatment, and F_3 is the emission intensity after 3 sec; the latter being the first identifiable signal on our recorder. These measurements exclude the usual "P" peak in fluorescence transients.

All samples were left in the dark for 10 min in the sample holder to settle before the experiment was started. The samples (thin suspension of algae) were under aerobic conditions, because their large surface-to-volume ratio allowed for a fast equilibration with atmospheric oxygen. Emission spectra were scanned with a speed of 1 mμ/sec and the excitation with 1.5 mμ/sec. Unless otherwise stated all measurements were performed at room temperature (22°C).

EXPERIMENTAL RESULTS

A. *Changes in Fluorescence Induced by 590 mμ Light Treatment*

Fig. 1 shows the fluorescence time course as a function of the duration of the light "treatment" at 590 mμ, where the first induction peak is not included. Orange light

(590 mμ) is absorbed mainly by phycocyanin (pigment system II). Chlorophyll *a* fluorescence (observed at 685 mμ) of nonpoisoned cells (*A*) rises rapidly within the first ½ min after the transition from dark to light. The initial rise is followed by a much slower rise (10 min), and then it declines until after about 60 min a steady level is reached. With DCMU-poisoned cells (*C*) the fast initial rise (within ½ min) followed by a slower rise lasts for about 20 min, and the ensuing decline continues throughout the time interval of observation (90 min). The per cent changes for poisoned samples are greater than those for the normal cells. In several experiments, F_t/F_3 in DCMU-poisoned cells rose to a value of 1.40. Curves *B* and *D* depict the phycocyanin fluorescence changes measured at 650 mμ for normal and DCMU-poisoned samples, with excitation and treatment at 580 mμ. Both curves have a slow

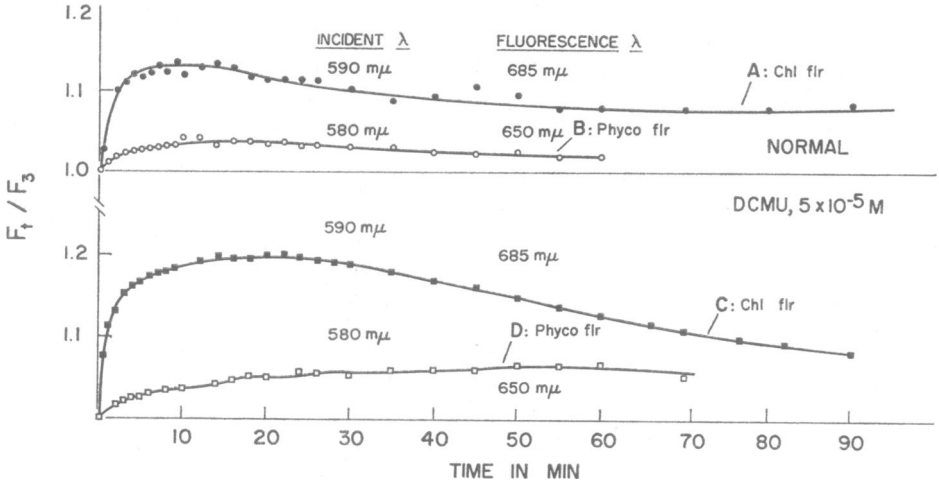

FIGURE 1 Time course of fluorescence of *Anacystis nidulans*, excited by orange light, in the minute range [fluorescence intensity at time $t(F_t)$/fluorescence intensity at 3 sec (F_3) vs. t].

initial rise followed by an extensive plateau. We believe that this small change is due mostly to fluorescence originating from phycocyanin since the wavelength of observation (650 mμ) is far removed from the Chl *a* emission maximum (685 mμ) and since the time course depicted in curves *B* and *D* are different from those in *A* and *C*, at least at longer times. Of course, it is possible that a small part of the observed change is due to Chl *a* fluorescence at 650 mμ. As in the case of Chl *a* fluorescence the per cent changes of the phycocyanin fluorescence of the poisoned sample are larger than those of the nonpoisoned.

Fig. 2 shows the emission spectra,[1] excited by 590 mμ for two different times of

[1] The spectra were not corrected for the variation of the fluorescence during the scanning interval because the time required to run the spectrum (about 50 sec) did not cause measurable changes; changes near 15 and 90 min are slow.

light treatment of normal and DCMU-poisoned *Anacystis nidulans*. Curves *A* and *D* correspond to spectra taken 15 min after the onset of treating illumination, while curves *B* and *E*, after 90 min. The chlorophyll *a* emission has decreased throughout the spectral region of observation. However, difference spectra[2] constructed by subtracting the emission values at 15 min (*F*15) from those at 90 min (*F*90) (curves *C* and *F*) show that there are two spectral regions within the Chl *a* red emission band where the changes are maximal, one at 685 mμ, the second at 693 mμ. This doublet

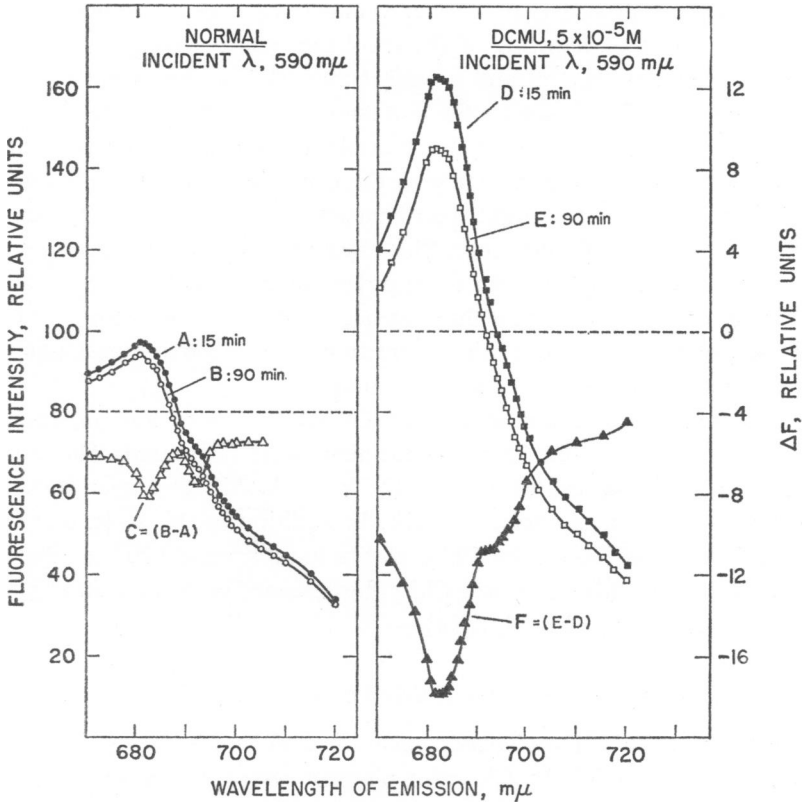

FIGURE 2 Emission and difference emission spectra of normal and DCMU-poisoned *Anacystis* after 15 and 90 min of illumination with orange light (590 mμ).

feature of the difference spectra has been observed repeatedly with cultures of varying ages, optical densities, and illumination intensities. The short-wavelength peak varied between 683 and 687 mμ, and the long-wavelength one fell between 692 and 700 mμ.

[2] In the case of DCMU-poisoned samples, the construction of the difference spectra was easy, but not so with normal cells, where the smaller magnitude of the changes, as well as the high proportion of phycocyanin fluorescence beyond 680 mμ, made the difference spectra less well-defined. The fact, however, that for normal cells, the difference curve given in Fig. 2 was observed in almost all (10) of the experiments performed, supports its validity.

Krey and Govindjee (17) reported that the emission spectrum of DCMU-poisoned *Porphyridium* cells has an additional hump at 692 mμ as compared with the emission spectrum of normal cells. In *Anacystis*, difference emission spectra (DCMU-poisoned minus normal) at 15, 30, and 60 min of illumination show a major peak at 683 mμ and a hump around 695 mμ (shown later in Fig. 4, right). The 695 mμ hump may perhaps be due to the same pigment complex that caused the 692 mμ hump in *Porphyridium*.

Fig. 3 (top) shows the excitation spectra of 745 mμ fluorescence of a nonpoisoned *Anacystis* sample measured after 15 min (*A*) and 75 min (*B*) of orange light (586 mμ) treatment. During the recording of the spectrum, which lasted for about 140 sec, the 586 mμ beam was cut off and a weak beam of 5 mμ bandwidth was used for excitation. Fig. 3 (middle) depicts the corresponding spectra of a DCMU-poisoned sample at 15 min (*C*) and 75 min (*D*) treatment, recorded in the same manner as above, while in the lower part of Fig. 3 the difference spectra are given. In both types of cells, it is clear that light treatment results in a reduced phycocyanin efficiency to sensitize Chl *a* fluorescence. This effect may be caused either by a lowering of the efficiency of energy transfer from phycocyanin to Chl *a* or by a decrease of the quantum efficiency of Chl *a* fluorescence itself (see Discussion). Phycocyanin fluorescence, which may have a minor contribution at 745 mμ, is assumed to be unchanged because of the shape of its time course.

The fluorescence excitation spectra of DCMU-poisoned and normal cells, as measured after 15 and after 75 min treatment, were compared. The curves were normalized at the phycocyanin peak (622.5 mμ). A shift to longer wavelengths is noticed (Fig. 4, left) in the poisoned cells. In addition to this shift, there seems to be an additional contribution by Chl *a* with a band around 670 mμ. The emission spectra show that DCMU increases Chl *a* fluorescence peak after 30 min and adds a minor peak at 698 mμ (Fig. 4, right).

B. *Changes in Fluorescence Induced by 440 mμ Light Treatment*

When normal or DCMU-poisoned *Anacystis nidulans* is exposed to blue light (440 mμ), absorbed mainly by Chl *a* (or pigment system I), the quantum efficiency of Chl *a* fluorescence changes with the duration of the blue light treatment. Fig. 5 shows the time course of these changes for fluorescence observed at 685 mμ, with a bandwidth of 26 mμ. The wide observation band was chosen in order to obtain a higher signal and also because excitation in the blue end of the absorption spectrum gives a relatively pure Chl *a* fluorescence. Curve *A* corresponds to the fluorescence time course of a normal sample; the light-induced fluorescence increase is relatively small (4%). In the case of poisoned cells (*B*) the changes are faster and greater in magnitude, amounting to 26%. A maximum steady level is reached after about 30 to 40 min of treatment. This feature distinguishes the blue light time course from those obtained with orange light since in the latter, the time course exhibited a broad maximum.

Fig. 6 shows the initial time course of fluorescence changes brought about by 440 mμ light treatment for two exciting and treating light intensities. Normal *Anacystis* shows in the first few seconds a fluorescence maximum which is followed by a decrease in fluorescence and then a steady rise to the emission intensity at

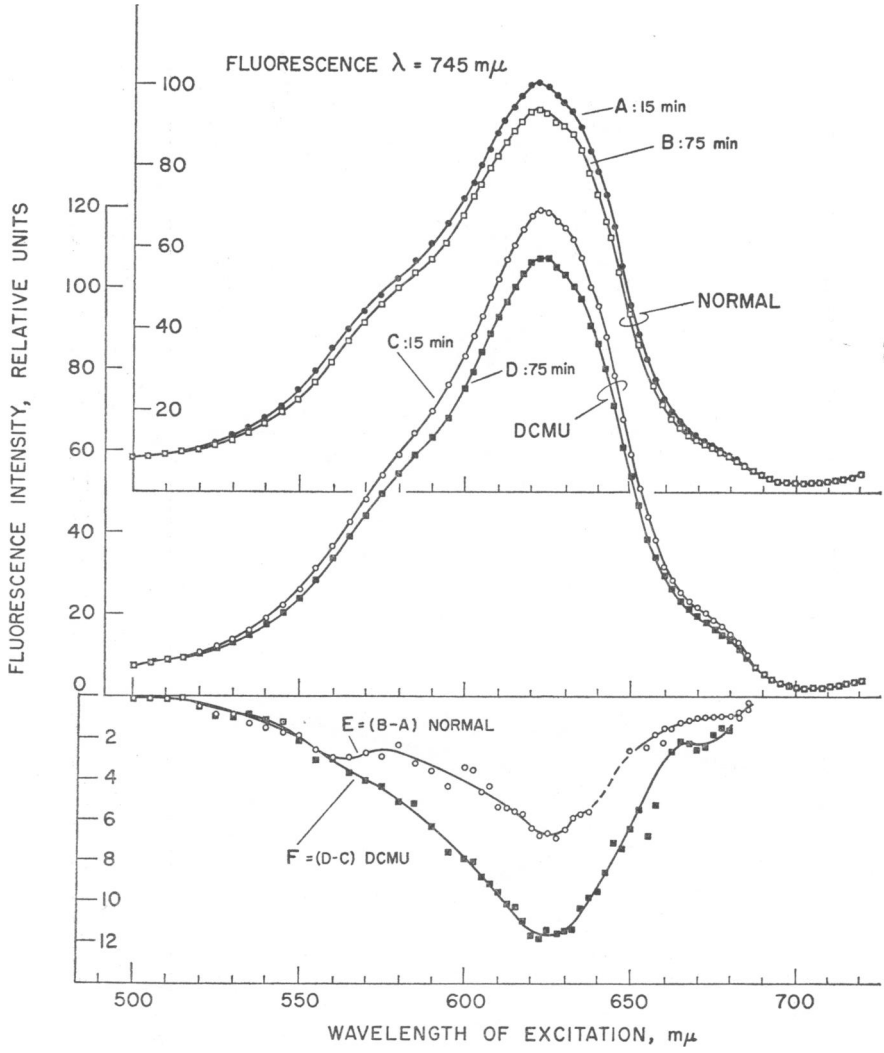

FIGURE 3 Excitation and difference excitation spectra of fluorescence of normal and DCMU-poisoned *Anacystis* after 15 and 75 min of light treatment.

longer times (top curves). Comparison of these curves (*A*, *B*) shows that the magnitude of the changes and possibly the rates increase with increasing light intensity. Poisoned *Anacystis* exhibits only an increase of the fluorescence amplitude as function of the duration of the blue light treatment (bottom curves). Both the magnitude and the rate of the changes depend on the incident treating light intensity.

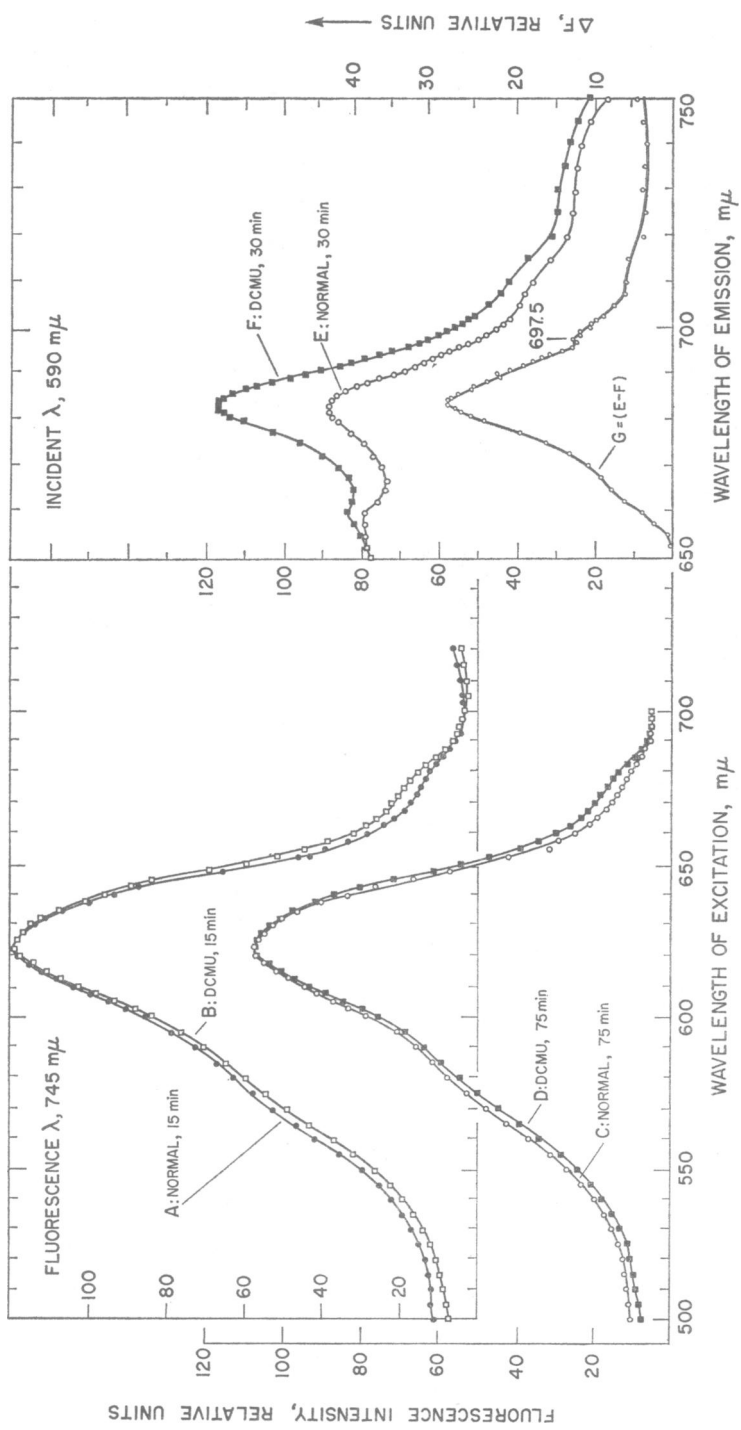

FIGURE 4 Excitation and emission spectra of DCMU-poisoned and normal cells.

The emission spectra of DCMU-poisoned *Anacystis* excited with 440 mμ at 5 min (*A*) and 60 min (*B*) of light treatment are shown in Fig. 7. Curve *C* is the difference emission spectrum (*F*60-*F*5). The doublet feature, observed earlier in the difference

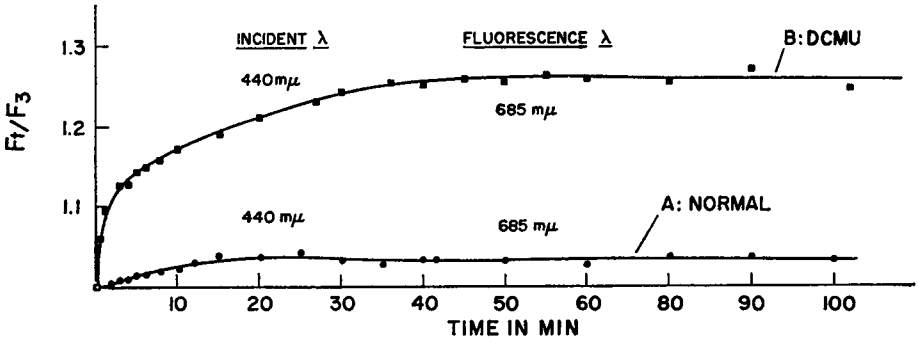

FIGURE 5 Time course of fluorescence of *Anacystis* excited by blue light in the minute range.

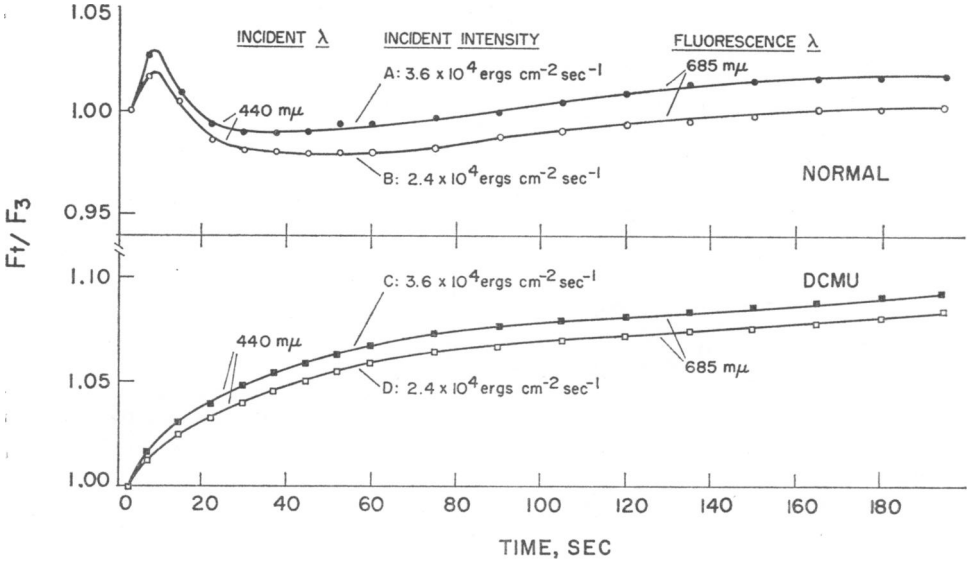

FIGURE 6 Time course of fluorescence of *Anacystis* excited by blue light at two different intensities.

spectrum with excitation and treatment at 590 mμ, is also apparent here. The difference maxima are located at 685 and 694 mμ. The increase in fluorescence emission of normal *Anacystis* cells due to 440 mμ treatment was too small to give an acceptable difference emission spectrum.

Absorption spectra of both poisoned and normal samples, measured before and after longtime illumination, did not show any significant difference. This confirms the results of Brody and Brody (3) on *Porphyridium cruentum.*

When normal and DCMU-poisoned samples were cooled to liquid nitrogen temperature (77°K) in the presence of either 440 or 590 mμ light, the fluorescence in-

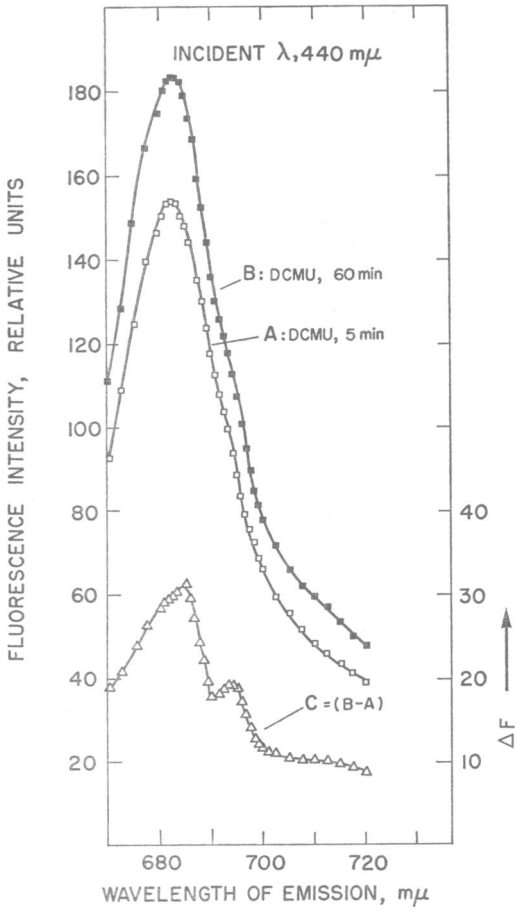

FIGURE 7 Emission spectra of DCMU-poisoned *Anacystis*, illuminated with blue light, at two different times.

tensity at 77°K remained constant with time under blue as well as green illumination. This also confirms the findings of Brody and Brody (3) on *Porphyridium cruentum.*

DISCUSSION

The experimental evidence presented here shows that prolonged treatment with light changes the fluorescence intensity of the blue-green alga *Anacystis nidulans.*

The color and the intensity of the treating light were of primary importance for the time course of these changes (see Table I). In addition, it is shown that the detailed course of events and the magnitude of the changes are different for normally photosynthesizing cells than for DCMU-poisoned ones.

The chlorophyll *a* fluorescence time courses are of similar shape for normal and DCMU-poisoned *Anacystis*. There are, however, differences in the first few seconds, and the timing and the magnitude of the events are changed. Whether the observed changes are due to a gradual shift of the chemical equilibria associated with the steady-state dark photosynthetic reactions or to a light-activated modification of the physical parameters, e.g. by changing the intermolecular distances which determine

TABLE I

FLUORESCENCE CHANGES INDUCED BY PROLONGED ILLUMINATION

Observable \ Light treatment and excitation	Orange light (590 mμ)		Blue light (440 mμ)	
	Normal cells	DCMU cells	Normal cells	DCMU cells
Maximum per cent increase at 685 mμ	13	20	4	26
Maximum per cent increase at 650 mμ	3.5	5	0	0
Location of fluorescence difference bands (*F*long time-*F*short time)	685 mμ 693 mμ	685 mμ 693 mμ	?	685 mμ 694 mμ
Location of excitation difference bands (*F*long time-*F*short time)	625 mμ	625 mμ	—	—
Location of fluorescence difference bands (*F*DCMU-*F*normal)	684 mμ	697.5 mμ	—	—

the excitation energy coupling between the pigment molecules, cannot be decided unambiguously on the basis of the existing evidence. It seems likely, however, that an accumulation of a reduced intermediate, Duysens' QH (18) or Kautsky's A_1 (19), either because of some inbalance in the rates of the primary photoreactions in normal cells or because photoreaction I is unable to reoxidize the reduced intermediate in poisoned cells would result in a unidirectional increase in fluorescence up to a maximum steady level. The fact that under system II illumination the changes are biphasic, consisting of an initial rise followed by a decrease of the fluorescence yield, may indicate that more than one process is involved.

Brody and Brody (3) have ascribed the changes in Chl *a* fluorescence emission and sensitization of *Porphyridium cruentum*, caused by light treatment, to photoactivated spatial rearrangements of the photosynthetic pigment molecules. In principle, a slight spatial reorientation will have no measurable effect on the absorption

properties, but can cause marked changes in the efficiency of both the homogeneous Chl *a* to Chl *a* transfer and the heterogeneous phycobilin to Chl *a* transfer. Both the intermolecular distance and the angle between the donor and acceptor oscillators are of importance for this transfer (20). However, photoactivated spatial rearrangements of the pigments, due presumably to conformational changes of the supporting lipoproteidic matrix, are likely to have an effect also on the intrinsic fluorescence quantum efficiencies, by enhancing or reducing concentration quenching. Conformational changes in chloroplasts induced by light were first noted by Packer (21) and have been reviewed by Good et al. (22). Indeed, the quantum efficiency of Chl *a* fluorescence in vivo is abnormally high if one takes into account the high pigment concentration in the photosynthetic organisms (18). We may assume, therefore, that as far as the Chl *a* fluorescence efficiency is concerned, the pigment exists in a labile distribution which by a slight photoactivated conformational change of the underlying lipoproteidic matrix can be converted to one with lower or higher emission efficiency. This hypothesis is supported by the fact that under conditions which prevent conformational changes, as for example by freezing the samples at liquid nitrogen temperature, no significant change in the emission was detected. Unfortunately, we have not been able to make direct experiments on the light-induced conformational changes in *Anacystis* because as yet we do not have any satisfactory equipment set up for this purpose.

The increase in fluorescence yield effected by blue light, which is absorbed primarily by Chl *a*, may reflect the transformation of some Chl *a* molecules to a more fluorescent form by suppressing concentration quenching through a light-activated conformational change of the lipoproteidic matrix. Light absorbed primarily by phycocyanin can also have the same effect via transfer to Chl *a*. However, since 590 mμ excitation of Chl *a* fluorescence is a sensitized type of emission, both the quantum efficiency of fluorescence and the degree of energy coupling between the phycocyanin and Chl *a* molecules are of importance.

Conformational changes which increase the quantum yield of fluorescence can result in the decrease of the energy transfer, e.g., by increasing intermolecular distances. The shape of the fluorescence time course with 590 mμ excitation and treatment is typical for two antagonistic processes. Although it is premature to postulate actual mechanisms by which the emission changes are realized, we feel that we can retain the working hypothesis that Chl *a* excitation, directly or via energy transfer from an accessory pigment, causes a rearrangement of the pigment molecules. This new distribution is characterized by an increased intrinsic quantum efficiency of chlorophyll *a* fluorescence and a decreased phycocyanin to chlorophyll *a* energy transfer efficiency. The fact that phycocyanin fluorescence remains virtually unchanged with 590 mμ treatment when that of Chl decreases may not contradict our hypothesis because one cannot invoke a strict reciprocity between phycocyanin emission and Chl *a* fluorescence sensitization.

DCMU-poisoned *Anacystis* shows greater changes in the emission intensities than

normal *Anacystis*. Since this poison blocks the electron transport in the chain of redox intermediates between the photosynthetic systems I and II (5, 18), the excess of excitation energy, part of which is dissipated by radiative emission, can induce larger effects as compared with the normally photosynthesizing cells. This is supported by the fact that higher incident intensities increase both the rate and the magnitude of the changes. The alternative, however, exists that the inhibitor (DCMU) is not bound on reaction centers exclusively, but on the lipoproteidic matrix as well (23). The physical properties of the lipoproteidic matrix are altered by such an association, and this may have an indirect effect on the fluorescence emission changes of chlorophyll *a*.

The 695 mμ Fluorescence Band. Brody and Brody (3) have shown that the absolute maximum of the emission change in light-treated *Porphyridium cruentum* is located at or near the maximum of the chlorophyll *a* emission band. By measuring the emission spectra in *Anacystis nidulans* with narrower half band widths (2.5 mμ) and constructing difference fluorescence spectra (see Fig. 2–4, 7), we have consistently observed two bands, one with a maximum at 685 mμ (chlorophyll *a* peak) and the other around 695 mμ. The 695 mμ band cannot be due to the phycocyanin fluorescence since its fluorescence remains unchanged in the time interval used.

The existence of more than one chlorophyll *a* form in vivo has been inferred on the basis of various experiments (18, 24–26). Analyses of the red absorption band (27–29) and the emission band (14, 16, 17, 30–35) of Chl *a* clearly show the complexity of Chl *a* in vivo. The Chl *a* absorption band seems to be the superposition of at least two bands, one with a maximum around 670–672 mμ (Chl *a* 670) and another one with a maximum around 680 mμ (Chl *a* 680). The fluorescence band also consists of at least two particular bands with emission maxima at about 685 and 696–698 mμ. It is probable that spectrally differentiable chlorophyll *a* forms correspond to different holochromes in vivo, since chlorophyll *a* forms can be isolated with various fractionation procedures (14, 36–43), and since their emission intensities depend in a characteristic manner on the temperature (14, 16, 17, 44). There is no unequivocal agreement as to the correspondence between the absorption and emission bands. Thus, the 698 mμ emission band is assigned by some authors to the fluorescence originating from the energy trap of the photosynthetic system II (45, 46), while by others to the weakly fluorescent bulk chlorophyll of system I (30, 31). Kok (47) suggested that the 698 mμ band may be due to the fluorescence of pigment molecules that "feed" energy to the energy trap, *P*700.

The long wavelength difference emission band around 695 mμ, which appears as a result of the light treatment of both normal and DCMU-poisoned cells is, in all likelihood, due to a Chl *a* holochromic form having an emission maximum around 693–698 mμ that changes differently than the main band (at 685 mμ). On the basis of Stoke's shift argument, this Chl *a* form can be assigned either to the system I bulk pigments or the system II trap pigments. The first assignment (to Chl a_1) is

supported by the arguments that the efficient chemical drainage of the trap excitation, the small energy separation between the bulk and trap lowest excited states, and their relative concentrations should not lead to an appreciable fluorescence from the traps. Also, light-induced conformational changes would affect preferentially the intrinsic fluorescence yield of the more populous bulk pigments than of the fewer isolated traps. The second assignment (to trap II) gains support from the fact that the 695 mμ difference band appears in the difference spectra between poisoned and nonpoisoned systems. Since in the former chemical de-excitation is absent, an excess of excited trap population may be formed which is reflected as the 695 mμ difference band (17). The existence of a 695 mμ fluorescence band is well established by a variety of experimental approaches. However, its exact origin remains largely undecided due to the chemical and spectroscopical complexity of the systems.

Note Added in Proof. New experiments indicate that the fluorescence yield of *Anacystis* declines to the 3 sec level when the excitation beam is cut-off. This may have an effect on the shape of the excitation spectra. In the case of emission spectra, however, the above-mentioned effect is of no consequence since their recording was done with the treating light as an excitation beam.

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